

CLAIMS

1. The use of a protein called sulfiredoxin (Srx),  
5 which comprises at least one catalytic site having  
the following motif: FXGCHR, with X = G or S, for  
catalyzing the reduction of peroxyredoxins (Prxs)  
in their superoxide form Prx-Cys<sub>p</sub>-SO<sub>2</sub>H  
(peroxyredoxin cysteine sulfinic acid) to a thiol  
10 derivative (SH).
2. The use as claimed in claim 1, characterized in  
that said sulfiredoxin is a sulfiredoxin of a  
microorganism, a plant or a higher organism, which  
15 generally comprises between 80 and 170 amino acids  
and at least the catalytic site having the  
following motif: FXGCHR, with X = G or S, and  
having the following percentage identities and  
similarities:  
20
  - yeast/human: 32% identity and 67% similarity
  - yeast/plants: 23% identity and 39% similarity
  - yeast/mouse: 31% identity and 51% similarity
  - yeast/fungi: 80% identity and 90% similarity.
- 25 3. The use as claimed in claim 1 or claim 2,  
characterized in that said sulfiredoxin is in  
particular selected from proteins whose sequences  
correspond, respectively, to the sequences SEQ ID  
No. 1 to 10.  
30
4. An isolated peptide corresponding to the catalytic  
site of Srx, as defined in claims 1 to 3,  
characterized in that it is defined by the  
following sequence; FXGCHR, with X = S.  
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5. A medicinal product, characterized in that it  
comprises an effective amount of a protein defined  
by a sequence selected from the group consisting

of the sequences SEQ ID No. 1-3 and 5-10, and, optionally at least one pharmaceutically acceptable excipient.

- 5    6.    The use of a protein as defined in claims 1 to 3,  
for preparing an antioxidizing medicinal product  
for use in the treatment of cancers,  
neurodegenerative disorders and neuromuscular  
diseases, in which a fault in the Prx/Srx  
10    antioxidizing system is observed.
7.    A method of screening for diseases related to  
cancer, to ageing, to neurodegenerative diseases  
and to neuromuscular diseases, which method is  
15    characterized in that it comprises, for evaluating  
the involvement of the Prx/Srx antioxidizing  
system:
- 20    (1) bringing the cells of a biological sample  
into contact, *in vitro*, with hydrogen  
peroxide ( $H_2O_2$ ),
- 25    (2) detecting the Prx-Cys<sub>P</sub>-SO<sub>2</sub>H formed, between 1  
hour and 4 hours after said bringing into  
contact according to step (1), and
- 30    (3) establishing the ratio of the amounts of  
Prx-Cys<sub>P</sub>-SO<sub>2</sub>H and of Prx-Cys<sub>P</sub>-SH, from 4 hours  
after said bringing into contact according to  
step (1).
- 35    8.    A method of screening for diseases related to  
cancer, to ageing, to neurodegenerative diseases  
and to neuromuscular diseases, which method is  
characterized in that it comprises genotyping of  
the sulfiredoxin, using the total RNA of a  
suitable biological sample, in particular blood  
cells, in accordance with the following steps:

- (1) extracting the total RNA from said biological sample,
  - (2) preparing specific sulfiredoxin cDNA by amplification of the RNA using the following two primers:  
GTCCCGCGGCGGCGGCGACG (SEQ ID No. 11)  
AGCAGGTGCCAAGGAGGCTG (SEQ ID No. 12),  
these sequences being located, respectively,  
upstream and downstream of the human sulfiredoxin ORF (GenBank No. AAH47707),
  - (3) establishing its nucleotide sequence, and
  - (4) comparing with respect to a DNA sequence encoding an Srx protein, as defined above, derived from the same species as that of the biological sample to be analyzed.
9. A method of screening for diseases related to cancer, to ageing, to neurodegenerative diseases and to neuromuscular diseases, which method is characterized in that it comprises relative quantification, by any appropriate means, of the mRNA encoding sulfiredoxin from the total cDNA prepared from a human biological sample, by comparison with a reference sample.
10. The method as claimed in claim 9, characterized in that said quantification comprises:
- (a1) preparing cDNA from the total RNA by reverse transcription with appropriate primers, and in particular random hexanucleotide primers;
  - (a2) amplifying said cDNA in the presence of the pair of primers:  
GTCCCGCGGCGGCGGCGACG (SEQ ID No. 11)  
AGCAGGTGCCAAGGAGGCTG (SEQ ID No. 12),

in the presence of a fluorescent reporter,  
and simultaneously or sequentially,

- 5 (a3) detecting the amount of the amplimer (or  
amplicon) by measuring the fluorescent  
signal.
11. The method as claimed in claim 10, characterized  
10 in that the fluorescent reporter is selected from  
the group consisting of agents that bind to  
double-stranded DNA and fluorescent probes.
12. The method as claimed in claim 10 or claim 11,  
15 characterized in that, when said fluorescent  
reporter is a probe, it is preferably selected  
from the group consisting of the probes defined by  
the following sequences:  
TTAATTGAATTCATGGGGCTGCGTGCAGGAGG (SEQ ID No. 13)  
20 and  
TTTTCCTTTTGCGGCCGCTACTACTGCAAGTCTGGTGTGGATG (SEQ  
ID No. 14).
13. A method of screening for diseases related to  
25 cancer, to ageing, to neurodegenerative diseases  
and to neuromuscular diseases, which method is  
characterized in that it comprises:  
- immunodetection of the Srx protein in a  
biological sample to be tested, using an  
30 antibody obtained by suitable immunization of  
an animal with an Srx protein or the peptide  
FXGCHR, with X = G or S, after separation of  
total proteins by electrophoresis, then  
- evaluation of the quality and of the amount of  
35 said Srx protein compared with a control Srx  
protein.
14. The use of the sequence coding for an Srx protein,  
as defined in claims 1 to 3, for obtaining plants

whose abilities to withstand stress are significantly increased.

- 5 15. Host cells, characterized in that they are transformed with a recombinant vector containing a sequence encoding an Srx protein, defined by a sequence selected from the group consisting of the sequences SEQ ID Nos. 1-3, 5, 6 and 8-10.
- 10 16. The host cell as claimed in claim 15, characterized in that it consists of an *S. cerevisiae* strain modified with a vector overexpressing the *SRX1* gene.
- 15 17. The host cell as claimed in claim 15, characterized in that it consists of a mammalian cell modified with a vector overexpressing the *hSrx1* gene.
- 20 18. The host cell as claimed in any one of claims 15 to 17, characterized in that said vector is advantageously an *E. coli*/*S. cerevisiae* shuttle vector comprising, at an *EcoRI* cloning site, the sequence encoding the Srx protein and the promoter of the *Srx* gene.  
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- 30 19. A method of screening for medicinal products capable of modulating the activity of the Prx/Srx antioxidizing system, characterized in that it comprises:
  - 35 (1) bringing the substance to be screened into contact with host cells as claimed in any one of claims 15 to 18, in the presence of hydrogen peroxide,
  - (2) detecting the Prx-Cys<sub>p</sub>-SO<sub>2</sub>H formed, between 1 hour and 4 hours after said bringing into contact according to step (1),

- 5 (3) establishing the ratio of the amounts of  
Prx-Cys<sub>P</sub>-SO<sub>2</sub>H and of Prx-Cys<sub>P</sub>-SH, from 4 hours  
after said bringing into contact according to  
step (1).
- 10 20. A method of screening for medicinal products that  
are useful in the treatment of cancers, of  
neurodegenerative diseases and of neuromuscular  
diseases, related to a fault in the Prx/Srx  
antioxidizing system, characterized in that it  
comprises:
- 15 a) bringing the substance to be tested into  
contact with an extract of host cells as  
claimed in any one of claims 15 to 18 or a  
biological sample of a nonhuman transgenic  
animal, in particular mice, selected from the  
group consisting of animals in which the gene  
20 of the Srx protein is knocked out and animals  
in which the gene of the Srx protein is  
overexpressed, in the presence of hydrogen  
peroxide,
- 25 b) measuring, by any appropriate means, the  
antioxidizing activity of the Prx/Srx system of  
the mixture obtained in a), and
- 30 c) selecting the substances capable of stimulating  
or of inhibiting said activity.
- 35 21. The method as claimed in claim 20, characterized  
in that the measurement of said activity is in  
particular carried out by detecting the Prx-  
Cys<sub>P</sub>-SO<sub>2</sub>H formed, between 1 hour and 4 hours after  
said bringing into contact according to step (a),  
and establishing the ratio of the amounts of Prx-  
Cys<sub>P</sub>-SO<sub>2</sub>H and of Prx-Cys<sub>P</sub>-SH, from 4 hours after  
said bringing into contact according to step (a).

22. A method of screening for medicinal products that are useful in the treatment of cancers, of neurodegenerative diseases and of neuromuscular diseases, related to a fault in the Prx/Srx antioxidizing system, characterized in that it comprises:
- (1) bringing the substance to be screened into contact with nonhuman transgenic mammals, in particular mice, selected from the group consisting of animals in which the gene of the Srx protein is knocked out and animals in which the gene of the Srx protein is overexpressed, and
- (2) measuring the survival of the animal.
23. Anti-Srx antibodies, characterized in that they are obtained by suitable immunization of an animal with an Srx protein defined by a sequence selected from the group consisting of the sequences SEQ ID No. 1-3, 5, 6 and 8-10 or the peptide FXGCHR, with X = S, as claimed in claim 4.
24. A method of reducing a product comprising at least two cysteines with redox activity, which method is characterized in that it comprises bringing said protein into contact with a sulfiredoxin (Srx), as defined in claims 1 to 3, which comprises at least one catalytic site having the following motif: FXGCHR, with X = G or S, in the presence of ATP and of magnesium.
25. A method of synthesizing a product comprising Cys-SH residues from products comprising Cys-SO<sub>2</sub>H residues, characterized in that it comprises a step consisting of reduction of the product comprising the Cys-SO<sub>2</sub>H residues to a product

comprising Cys-SH residues, in the presence of a sulfiredoxin as defined in claims 1 to 3, of ATP and of magnesium.